PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: P. A. Billing-Medel, *et al.*

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For: REAGENTS AND METHODS USEFUL FOR DETECTING THE BREAST

Examiner: L. Arthur

Group Art Unit: 1655

Case No.: 5995.US.P1

Date:

CERTIFICATE OF MAILING (37 CFR 1.8 (a))

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the:

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Wanda C. Smith 4/25/02
Wanda E. Smith Date

DECLARATION OF
PAULA N. FRIEDMAN Ph.D.

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

1. I am one skilled in the art of cancer diagnostics. I have a Ph.D. in Molecular Biology from Columbia University as well as an M.A. and a M. Phil. in Molecular Biology also from Columbia University. I further have a B.A. in Biology from Dartmouth College.

2. I was a Postdoctoral Fellow in the Laboratory of Dr. Clay Siegall at the Pharmaceutical Research Institute Bristol–Myers Squibb and an Assistant Pharmacologist, Dept. of Clinical Immunology & Biol. Therapy at the MD Anderson Cancer Center.

3. I have nine years of research and development experience in the cancer diagnostic industry. Much of my work has involved the discovery and validation of novel cancer markers to improve the accuracy of diagnosing the onset of cancer. In fact, I am a named inventor of several U.S. Patents, all of which are related to the field of cancer diagnostics.

4 I also have authored numerous journal articles relating to cancer pathology, detection, and metastasis (see Attachment I).

5. I am one of the named inventors of the aforementioned application.

6. I have read and am familiar with the Patent Office Action dated August 28, 2001 and utility rejection under 35 U.S.C. 101 applied against the present application.

7. At my direction, Dr. Tim Stenzel in the Department of Pathology at Duke University in Chapel Hill, North Carolina, conducted an RT-PCR assay on lymph node tissue from either breast cancer patients or non-breast cancer patients. RNA was isolated from the lymph node tissues using the Qiagen RNeasy kit and then subjected to quantitative RT-PCR using primers specific for the BS106 gene. The BS106 product was quantitated by comparing the values to a standard curve of SKBR3 (breast cancer cell line) RNA. The purpose of this experiment was to show that the BS106 gene is expressed in breast cancer cells that have escaped the primary tumor. The RT-PCR assay, like the one described here, is useful in distinguishing lymph nodes that contain cancer cells from those that do not. Dr Tim Stenzel's lab at Duke is a leading laboratory that searches for new molecular tests that can help doctors more accurately stage breast cancer patients and therefore provide their patients with the best possible care.

8. The results of the BS106 RT-PCR assay are shown in Attachments A and B. Attachment A shows the quantitative RT-PCR results for the nodes from breast cancer patients. All of the values for the 9 samples are positive indicating that there are breast cells present. Some nodes have more cells then others, resulting in the higher values. Attachment B shows the results for the non-breast cancer nodes and one can see that all these values are zero except for one very low positive sample (ABNLLN 19). Below each table is a summary of the data. These results indicate that BS106 is detected in 9/9 cancer nodes and 1/20 normal nodes. This is a sensitivity of 100% and a specificity of 95% for the detection of metastatic cells in the lymph nodes.

9. The results in Paragraph 8 confirm that BS106 can be used as a marker for the detection of breast cells in the lymph nodes that have escaped the primary tumor.

10. I hereby declare that all statements made herein are of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States code and such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Paula N. Friedman, Ph.D.

4/24/02
Date

ATTACHMENT I

Publications:

Wang, E.H., **Friedman, P.N.** and Prives, C. 1989. The murine p53 protein blocks replication of SV40 DNA in vitro by inhibiting the initiation functions of SV40 large T antigen. Cell, 57, 379-392.

Manfredi, J.J., Wang, E.H., **Friedman, P.N.,** and Prives, C. 1989. Purified SV40 large T antigen in complex with murine p53 does not support SV40 DNA replication in vitro. Implications for the mechanism of transformation by SV40 large T antigen. In Common Mechanisms of Transformation by Small DNA Tumor Viruses. Luis P. Villeareal, ed. American Society for Microbiology,113.

Bischoff, J.R., **Friedman, P.N.,** Marshak, D., Prives, C., and Beach, D. 1990. The p53 protein is phosphorylated by cyclin A-cdc2 as well as cyclin B-cdc2. PNAS, 87,4766-4770.

Friedman, P.N., Kern, S.E., Vogelstein, B., and Prives, C. 1990. Wild-type, but not mutant, human p53 proteins inhibit the replication activities of simian virus 40 large tumor antigen. PNAS, 87, 9275-9279.

Kern, S.E., Kinzler, K.W., Baker, S.J., Nigro, J.M., Rotter,V., Levine, A.J., **Friedman, P.N.,** Prives, C. and Vogelstein, B. 1991. Mutant p53 proteins bind DNA abnormally in vitro. Oncogene, 6, 131-136.

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Farmer, G., Bargonetti, J., Zhu, H., **Friedman, P.N.,** Prywes, R., and Prives, C. 1992. Wild-type p53 activates transcription in vitro. Nature, 358, 83-86.

Prives, C., Bargonetti, J., **Friedman, P.N.,** Manfredi, J.J., and Wang, E. 1992. Functional consequences of the interaction between the p53 tumor suppressor protein and the SV40 large tumor antigen. Cold Spring Harbor Symposium on Quantitative Biology: The Cell Cycle, Vol. 56, 227-235.

Bargonetti, J., Reynesdottir, I., **Friedman, P.N.,** and Prives, C. 1992. Wild-type p53 site-specific binding to cellular DNA is regulated by SV40 T antigen and mutant p53. Genes and Devel., 6, 1886-1898.

Friedman, P.N., Wang, E.H., Meerovitch, K., Sonenberg, N., and Prives, C. 1992. Murine p53 inhibits the function but not the formation of SV40 T antigen hexamers and stimulates T antigen RNA helicase activity. Chromosoma, 102, 60-66.

Friedman, P.N., Chen, X., Bargonetti, J., and Prives, C. The p53 protein is an unusually shaped tetramer that binds directly to DNA. PNAS, 90, 3319-3323.

Reynesdottir, I., Lorimer, H.E., **Friedman, P.N.,** Wang, E.H., and Prives, C. 1993. Phosphorylation and active ATP hydrolysis are not required for SV40 T antigen hexamer formation. J. of Biol. Chem., 268, 24647-24654.

Friedman, P.N., McAndrew, S.J., Gawlak, S.L., Chace, D., Trail, P.A., Brown, J.P., and Siegall, C.B. 1993. BR96 sFv-PE40, a potent single-chain immunotoxin that selectively kills carcinoma cells. Cancer Res., 53, 334-339.

Friedman, P.N., Chace, D.F., Trail, P.A., and Siegall, C.B.1993. Antitumor activity of the single-chain immunotoxin BR96 sFv-PE40 against established breast and lung tumor xenografts. J. of Immun., 150, 3054-3061.

Friedman, P.N., Chace, D.F., Gawlak, S.L., and Siegall, C.B. 1993. The single-chain immunotoxins BR96 sFv-PE40 and BR96 sFv-PE38: Potent anti-tumor agents for the treatment of human cancer. In Growth Factors, Peptides, and Receptors, T. Moody, ed., Plenum Press, 409-414.

Attachment A

Lymph nodes from breast cancer patients	SKBR3 Ng equivalents					Ratio of Marker/ Beta 2 Micro			
Samples	Du101	DS106	Mamma	Cyto	Beta2	Du101/Beta2	DS106/Beta2	Mamm/Beta2	Cyto/Bet
ABNLLN21 LN CA	2E0.0000	31 0.00	1 10.0L00	45 0.000	10L.0000	2.6.00L	L.31U0	1.1000	0.45LL
ABNLLN22 LN CA	E.3000	1000.00C0	0.0490	32 0000	82.000C	0.10'2	12.1951	0.0006	0.39C2
ABNLLN23 LN CA	E.9000	5900.00C0	1.50C0	32 0000	87.000C	0.1023	67.3161	0.0'72	0.367E
ABNLLN28 LN CA	1.3000	30 000C	0.0'30	53 0000	74.000C	0.017E	C.4C54	0.0002	0.71E2
ABNLLN29 LN CA	1.1000	4700.00C0	0.0270	13 0000	13C.0000	0.0085	36.1538	0.0002	0.10CC
ABNLLN30 LN CA	14.00C0	3.0000	0.39C0	16 0000	35.000C	0.400C	0.03571	0.0'11	0.4571
ABNLLN31 LN CA	20.00C0	29 000C	39.0000	85 0000	0.3100	64.5161	93.5484	125.8065	274.193
ABNLLN34 LN CA	E.9000	98 000C	3.10C0	9.30C0	6.3000	1.0952	15.5556	0.4921	1.47E2
ABNLLN44 LN CA	C.0000	2.5000	0.52C0	7.40C0	90.000C	0.0422	C.027.0	0.0069	0.0022
Number of Positive Samples	(9/9)	(9/9)	(9/9)	(9/9)	(9/9)				
% Fositve Samples	100%	100%	100%	100%	10C%				

I N CA =lymph nodes with histological cancer

Attachment B

Lymph nodes from non-cancer patients	SKBR3 Ng equivalents					Ratio of Marker/ Beta 2 Micro			
Samples	Du101	DS106	Mamma	Cyto	Beta2	Du101/Beta2	DS106/Beta2	Mamm/Beta2	Cyto/Bet
ABNLLN 2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
ABNLLN 3	[illegible]	0.0000	0.0000	0.0013	83.0000	0.0000	0.0000	0.0000	1.5663E-
ABNLLN 4	[illegible]	0.0000	0.0000	0.0000	12.0000	0.0000	0.0000	0.0000	0.0000
ABNLLN 5	[illegible]	0.0000	0.0000	0.0000	6.1000	0.0000	0.0000	0.0000	0.0000
ABNLLN 6	[illegible]	0.0000	0.0000	0.0000	16.0000	0.0000	0.0000	0.0000	0.0000
ABNLLN 8	[illegible]	0.0000	0.0000	0.0000	51.0000	0.0000	0.0000	0.0000	0.0000
ABNLLN 9	[illegible]	0.0000	0.0000	0.0000	26.0000	0.0000	0.0000	0.0000	0.0000
ABNLLN 10	[illegible]	0.0000	0.0000	0.0000	69.0000	0.0000	0.0000	0.0000	0.0000
ABNLLN 11	[illegible]	0.0000	0.0000	0.0000	06.0000	0.0000	0.0000	0.0000	0.0000
ABNLLN 12	[illegible]	0.0000	0.0000	0.0000	13.0000	0.0000	0.0000	0.0000	0.0000
ABNLLN 13	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
ABNLLN 14	[illegible]	0.0000	0.0000	0.0000	71.0000	0.0000	0.0000	0.0000	0.0000
ABNLLN 15	[illegible]	0.0000	0.0000	0.0000	120.0000	0.0000	0.0000	0.0000	0.0000
ABNLLN 16	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
ABNLLN 17	[illegible]	0.0000	0.0000	0.0000	21.0000	0.0000	0.0000	0.0000	0.0000
ABNLLN 18	[illegible]	0.0000	0.0000	0.0000	47.0000	0.0000	0.0000	0.0000	0.0000
ABNLLN 19	[illegible]	0.0720	0.0000	0.0011	130.0000	0.0000	0.0005	0.0000	8.4615E-
ABNLLN 48	[illegible]	0.0000	0.0000	0.0000	5.5000	0.0000	[illegible]	0.0000	0.0000
ABNLLN 49	[illegible]	0.0000	0.0000	0.0000	30.0000	0.0000	[illegible]	0.0000	0.0000
ABNLLN 50	[illegible]	0.0000	0.0000	0.0000	11.0000	0.0000	[illegible]	0.0000	0.0000
Number of Positive Samples	(0/20)	(1/20)	(0/20)	(3/20)	(20/20)				
% Positive Samples	0%	5%	0%	15%	100%				